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ORIGINAL PAPER

Localisation of the gene for a dominant congenital spinal muscular atrophy predominantly affecting the lower limbs to chromosome 12q23–q24

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Spinal muscular atrophies are a heterogeneous group of disorders. They differ in time of onset, clinical presentation, progression, severity and mode of inheritance. In 1985 a Dutch family was described with a dominant, non-progressive spinal muscular atrophy presenting at birth with arthrogryposis (MIM 600175). Linkage analysis was performed in this family. After having excluded the loci for Werdnig-Hoffmann's disease and for dominant distal spinal muscular atrophy with upper limb predominance, we were able to localise the gene to a 10 cM interval between the markers *D12S78* and *D12S1646* on chromosome 12q23–q24. Recently, dominant scapuloperoneal spinal muscular atrophy has been localised to an overlapping interval. However, the clinical appearances of scapuloperoneal spinal muscular atrophy and the present disorder make allelism unlikely. In 1994, a second Dutch family with a disorder similar to the present one was described. We excluded linkage to markers of the 12q23–q24 region in this family and thereby proved genetic heterogeneity of this type of dominant, congenital and nonprogressive spinal muscular atrophy.

Keywords: spinal muscular atrophy (SMA); 12q23–24; linkage

Introduction

The spinal muscular atrophies (SMAs) are a rather large and heterogeneous group of disorders.[1–8] The most extensively studied condition in this group is autosomal recessive spinal muscular atrophy type I or Werdnig-Hoffmann's disease.[4] The primary defect in SMA lies in the lower motor neurons or anterior horn cells, which in some cases is shown by autopsy. A subset of SMA presents at birth with contractures and muscle atrophy,[1,5,7] suggesting a defect in the development of the anterior horn cells or early degeneration.[5] Therefore, the most likely time of onset is during or shortly after the third month of pregnancy, which is the critical period for differentiation of the spinal motor neurons.[9]

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A family with dominant, congenital SMA accompanied by contractures was described by Fleury and Hageman.[5] The condition in this family is nonprogressive and inherited in a dominant pattern. The mildly affected family members suffer from congenital weakness of the distal parts of the lower limbs only. More severely affected individuals show additional weakness of the pelvic girdle and truncal muscles, resulting in scoliosis. Tendon reflexes are absent or reduced and sensory abnormalities are not found. A similar type of dominant SMA has recently been described by Frijns *et al*.[7] The condition in both families cannot be categorised in the generally accepted classification system of Harding[10] due to its congenital nature. The disorder described by Fleury and Hageman can be uniformly classified as a congenital distal type. In the family described by Frijns *et al* one of the patients showed involvement of the scapular muscles.

We performed linkage analysis in the family described by Fleury and Hageman.[5] Both the loci for a distal form of dominant spinal muscular atrophy with upper limb predominance on 7p[11] and Werdnig-Hoffmann's disease on 5q12–q13[12,13] were excluded. We localised the gene for the SMA in the present family to 12q23–q24. The critical region overlaps with the interval between the markers *D12S338* and *D12S366* in which dominant scapuloperoneal spinal muscular atrophy (SPSMA) is located.[14] The critical region for the present SMA in 12q23–q24 was excluded in the family described by Frijns *et al*.[7]

Materials and Methods

Patients

For a detailed description of the patients suffering from the present dominant SMA we refer to the paper of Fleury and Hageman.[5] The authors discriminated four categories of severity. These varied from category one with slight paresis of foot extension and deformities of the feet to category four with paralysis of the distal part of the legs and serious paresis of the muscles of the trunk, the pelvic girdle and the proximal part of the legs. Additional investigations such as electromyography, investigation of motor nerve conduction velocities and muscle and nerve biopsies, showed evidence for a pathology of lower motor neurons. Blood samples were obtained from 20 members of this family,[5] 10 of whom were affected. Although individual IV-8 was marked as affected by Fleury and Hageman, reinvestigation did not confirm this. Her tendon reflexes and the strength in her feet are normal. Two non-affected individuals, III-18 and the son of III-20, have been tested for markers derived from the critical region only. From the family described by Frijns *et al*[7] blood samples were obtained from seven affected members is three generations, two non-affected members and two spouses.

Typing of DNA Markers

DNA was extracted using the method described by Miller *et al*.[15] Amplification of locus-specific DNA-fragments and their analysis was performed according to Kremer *et al*.[16]

Statistical Analysis of Linkage Data

Two-point lod scores were calculated, using the subroutine Mlink of the Linkage 5.1 package.[17–19] A gene frequency of 0.00001 and a penetrance of 100% were assumed for the disorders. Calculations of maximum lod scores were performed using the subroutine Ilink of the Linkage 5.1 package. Multipoint analysis was performed using five-point linkage analysis (Fastlink version 2.30)[20,21] combined with the sliding window technique.

Results

Linkage to Chromosome 12q23–q24

Linkage analysis was performed in the Dutch family described by Fleury and Hageman (family 1, Figure 1).[5] Twenty members of this family were reinvestigated and included in the DNA analysis. Three loci involved in different forms of SMA were regarded as candidates for the present disorder: 7p[11], 5q12–q13[12,13] and 12q24.1–q24.31.[14]

Chromosome 7p harbours a locus for a distal form of SMA. The markers D7S513, D7S493, D7S516, D7S526 and GCK, derived from this locus[11] made this location highly unlikely (Table 1). Since the proximal border of the critical region was not determined by Christodoulou *et al*,[11] we tested EGFR and D7S440 which excluded the centromeric region of chromosome 7. The chromosomal region involved in Werdnig-Hoffmann's disease (5q12–q13) was excluded using the polymorphic markers D5S407, D5S629 and D5S118 (Table 1). Subsequently, an indication for linkage was obtained with the marker *D12S79*, derived from the critical region described for SPSMA. The maximum lod score was 1.32 at $\theta = 0.08$. The markers *D12S366*, *D12S349* and *D12S1639* located distal to *D12S79*, showed a decrease of the lod score. Therefore, we started our fine mapping with polymorphisms located proximal to *D12S79*.

Fine Mapping

Nineteen polymorphic markers were tested derived from the region proximal to *D12S79*. Two-point lod scores between the relevant markers and the disease locus are given in Table 2. A maximum lod score of 5.16 at $\theta = 0.00$ was obtained with the marker *D12S1583*. In order to visualise the borders of the critical region haplotypes were constructed (Figure 1). The proximal border is determined by a recombination visible in

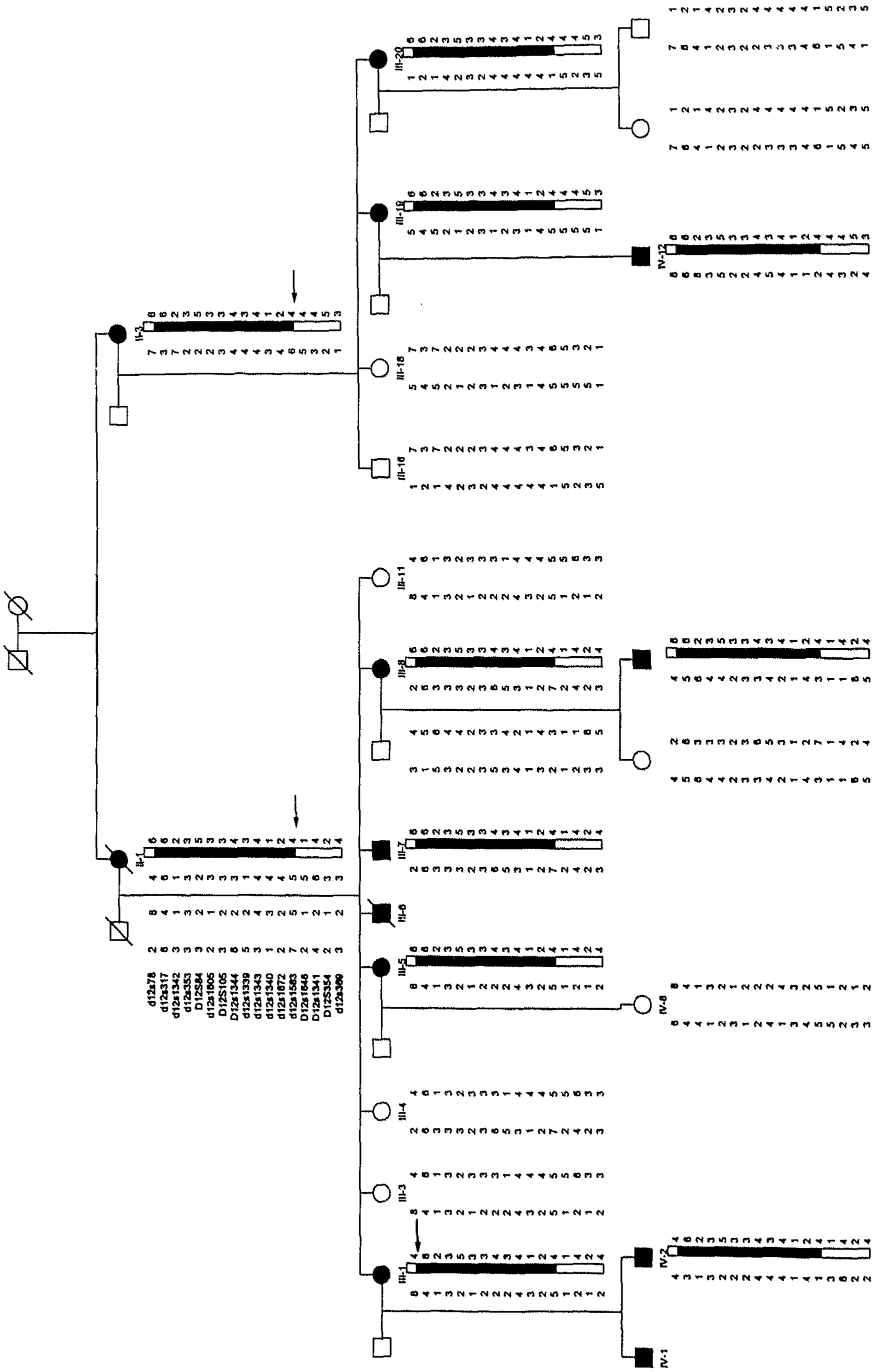

Figure 1 *Pedigree of family 1 and haplotypes of individuals available for this study. Individuals are numbered according to Fleury and Hageman.*[5] *Bars depict the chromosome carrying the mutation. The black part of the bars represents the common disease haplotype and arrows indicate crossovers.*

Table 1 Two-point lod scores for the candidate loci on chromosomes 5q and 7 in family 1

family 1	θ					
Locus	*0.00*	*0.01*	*0.05*	*0.10*	*0.20*	*0.30*
D5S407	−∞	−5.35	−2.08	−0.68	0.02	0.22
D5S629	−∞	−3.48	−1.58	−0.90	−0.34	−0.11
D5S118	−∞	−4.98	−2.20	−1.16	−0.34	−0.07
D7S513	−∞	−9.04	−4.34	−2.48	−0.92	−0.28
D7S493	−∞	−4.99	−2.95	−1.98	−0.88	−0.33
D7S516	−∞	−3.20	−1.82	−1.22	−0.59	−0.24
D7S526	−∞	−5.62	−2.33	−1.09	−0.14	0.16
GCK	−∞	−4.75	−2.69	−1.84	−1.02	−0.52
EGFR	−∞	−6.96	−3.56	−2.19	−0.98	−0.41
D7S440	−∞	−5.34	−3.15	−2.18	−1.21	−0.66

Maker order is derived from Dib *et al*[27].

individual III.1 between the markers *D12S78* and *D12S1342*. The marker *D12S317* is not informative for this recombination. The distal border of the critical region is determined by a recombination between the markers *D12S1583* and *D12S1646*. Different alleles of the marker *D12S1646* co-segregate with the disorder in the two branches of the pedigree. Although the marker *D12S1341* is located distal to *D12S1646* it has a high lod score which is due to non-informativity of this marker between the two branches of the pedigree (Figure 1). Five-point linkage analysis was performed to determine the location of the gene involved with the highest likelihood. A maximum lod score of 5.41 was found in the interval between D12S1339 and D12S1343 and in the interval between D12S1340 and D12S1672 (Figure 2). Both these regions are located between D12S1344 and D12S1583.

The SMA in the family described by Frijns *et al* (family 2),[7] closely resembles the disorder in family 1. Both families originated from the same geographical area. Therefore we analysed family 2 for linkage with markers derived from the interval between *D12S78* and *D12S354*. In Table 3 the two-point lod scores are given. These lod scores exclude the interval determined in family 1.

Discussion

In the present study we localised the gene responsible for congenital distal spinal muscular atrophy in family 1,[5] to an interval of about 10 cM in 12q23–q24, delimited by the markers *D12S78* and *D12S1646*. However, in family 2 linkage to this interval was excluded.

The disorders in family 1 and family 2[7] are clinically very similar (Table 4). Some of the patients of family 2 show a slight hyperlaxity of the upper limb joints and a mild weakness of the neck flexors. These findings are

Table 2 Two-point lod scores between the polymorphic markers derived from chromosome 12 and the SMA in family 1

family 1	θ						
Locus	*0.00*	*0.01*	*0.05*	*0.10*	*0.20*	*0.30*	Z_{max} (θ)
D12S78	−∞	2.79	3.17	3.06	2.48	1.69	3.17 (0.05)
D12S317	1.95	1.91	1.74	1.52	1.08	0.63	1.95 (0.00)
D12S1342	4.96	4.88	4.54	4.11	3.17	2.12	4.96 (0.00)
D12S353	1.99	1.96	1.80	1.61	1.20	0.76	1.99 (0.00)
D12S84	4.95	4.87	4.53	4.11	3.17	2.14	4.95 (0.00)
D12S1605	1.18	1.15	1.04	0.89	0.60	0.32	1.18(0.00)
D12S105	1.04	1.02	0.94	0.84	0.63	0.42	1.04 (0.00)
D12S1344	3.47	3.41	3.16	2.85	2.17	1.43	3.47 (0.00)
D12S1339	4.80	4.71	4.37	3.93	2.98	1.95	4.80 (0.00)
D12S1343	1.04	1.02	0.94	0.84	0.63	0.41	1.04 (0.00)
D12S1340	3.57	3.50	3.20	2.82	1.99	1.11	3.57 (0.00)
D12S1672	3.88	3.81	3.53	3.17	2.40	1.56	3.88 (0.00)
D12S1583	5.16	5.05	4.72	4.27	3.29	2.19	**5.16** (0.00)
D12S1646	−∞	0.77	1.89	2.11	1.90	1.34	2.12 (0.11)
D12S1341	4.36	4.29	3.98	3.57	2.69	1.72	4.36 (0.00)
D12S354	−1.51	1.60	2.01	1.94	1.44	0.80	2.02 (0.06)
D12S369	−∞	0.20	1.29	1.50	1.26	0.77	1.50 (0.11)
D12S79*	−∞	0.81	1.29	1.31	1.03	0.64	1.32 (0.08)
D12S366*	−∞	−6.45	−3.05	−1.68	−0.51	−0.06	0.07 (0.41)
D12S349*	−∞	−5.71	−2.42	−1.17	−0.22	0.06	0.08 (0.35)
D12S1639	−∞	−5.81	−2.51	−1.28	−0.30	0.03	0.08 (0.37)

*Individuals III–18 and the son of III–20 are not involved in the analysis.
Marker order, given from centromere to telomere, is derived from Kucherlapati *et al*[25] and Dib *et al*[27]

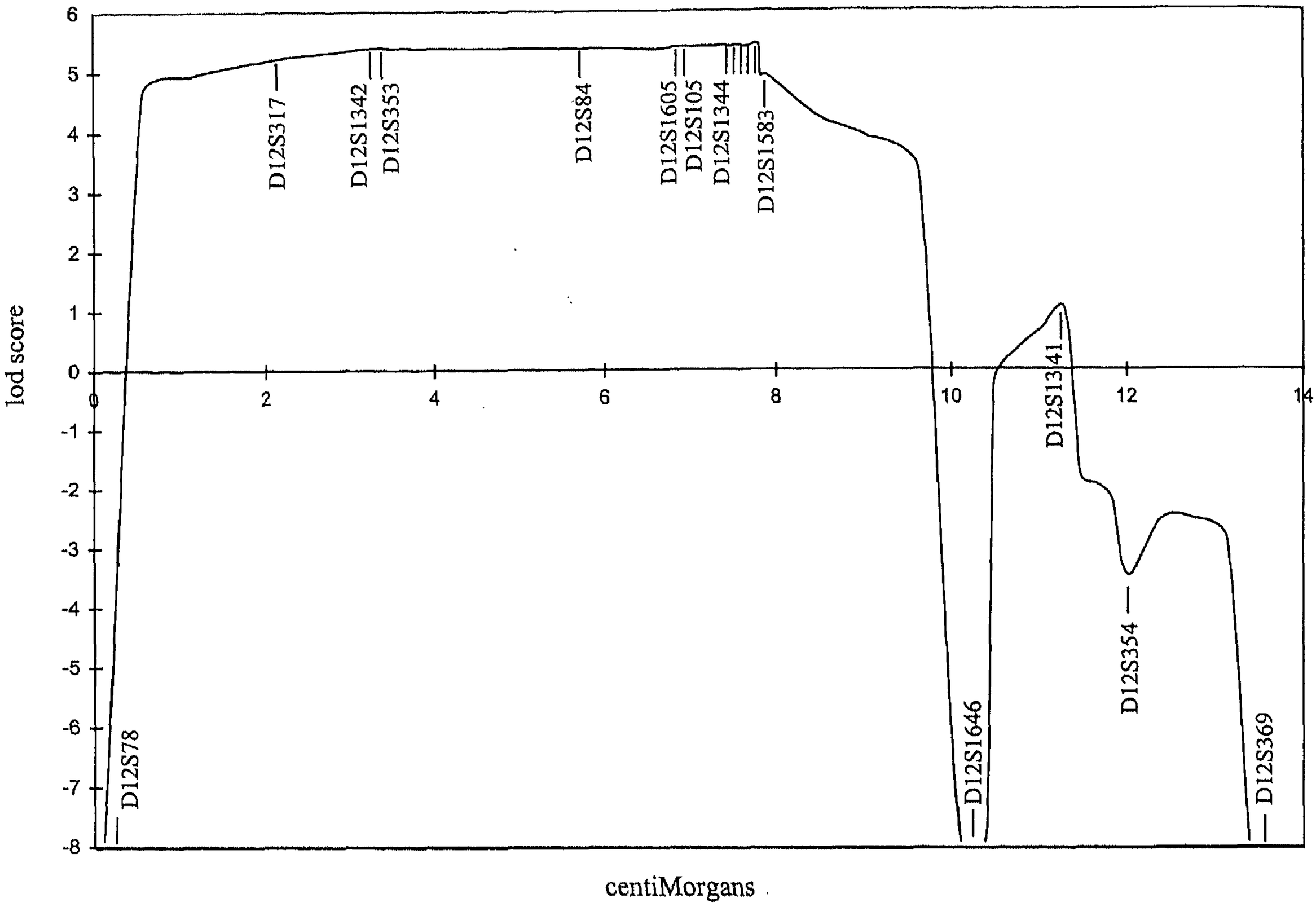

Figure 2 *Five-point linkage analysis combined with the sliding window technique resulting in a maximum lod score of 5.41 in the interval between* D12S1339 *and* D12S1343 *and in the interval between* D12S1340 *and* D12S1672. *The markers located between* D12S1344 *and* D12S1583 *represented by the lines are, from left the right,* D12S1339, D12S1343, D12S1340 *and* D12S1672.

not present in the patients of family 1. McKusick presents both disorders under the same MIM number (MIM 600175). Our results indicate genetic heterogeneity for this type of SMA.

Interestingly, the locus for the type of spinal muscular atrophy in family 1 has overlap with the locus for SPSMA,[14] which has been mapped to the 19 cM interval between *D12S338* and *D12S366* (Figure 3). However, clinically the two disorders are different (Table 4). At birth, the most remarkable feature of this autosomal dominant form of SPSMA is stridurous breathing due to laryngeal palsy. Lower limb weakness and contractures appear in the first or second decade only, indicating a progressive course of the disease. In contrast, the SMA in family 1 presents with contractures at birth, is limited to the lower extremities and is not progressive. Therefore, allelism of both disorders does not seem very likely. If they are allelic, the critical region for the gene involved is determined by the markers *D12S338* and *D12S1646*. The locus for distal

Table 3 Two-point lod scores between the polymorphic markers derived from chromosome 12q23–q24 and the disorder in family 2

family 2	θ					
Locus	*0.00*	*0.01*	*0.05*	*0.10*	*0.20*	*0.30*
D12S78	−∞	−2.80	−1.44	−0.89	−0.39	−0.15
D12S317	−∞	−3.91	−1.88	−1.08	−0.38	−0.08
D12S84	−∞	0.02	0.02	0.02	0.02	0.01
D12S1344	−∞	−1.11	−0.44	−0.19	0.01	0.07
D12S1339	−∞	−3.91	−1.88	−1.08	−0.38	−0.08
D12S1583	−∞	−3.91	−1.88	−1.08	−0.38	−0.08
D12S1646	−∞	−2.71	−1.36	−0.82	−0.34	−0.13
D12S354	−∞	−4.21	−2.16	−1.33	−0.58	−0.23

Maker order, given from centromere to telomere, is derived from Kucherlapati *et al*[25] and Dib *et al*[27].

Table 4 Clinical; comparison of scapulo peroneal spinal muscular atrophy (SPSMA)[6] and the disorders described by Fleury and Hageman[5] and Frijns *et al*[7]

	SPSMA	*Family 1 by Fleury and Hageman*	*Family 2 by Frijns et al*
mode of inheritance	autosomal dominant	autosomal dominant	dominant
age of onset	birth	birth	birth
signs at birth	stridurous respiration, congenital absence of muscle groups, legs not heavily involved	weakness and congenital contractures in lower limbs	weakness and congenital contractures in lower limbs
involvement	larynx, neck, scapular musculature and lower extremities	lower extremities and truncal muscles	lower extremities and paraspinal muscles, in some: masseteric, neck, shoulder girdle and upper limb musculature
progression	+ (mainly in distal parts of extremities)	–	–
deep tendon reflexes	absent	reduced to absent	reduced to absent
sensory	absence of vibration sense at 256 Hz	intact	intact
motor	progression to atrophied and paretic lower limb muscles	paretic proximal lower limbs and pelvic girdle, paralytic distal lower limbs	paresis of lower limbs and and lumbar paraspinal muscles
anticipation	+	–	–

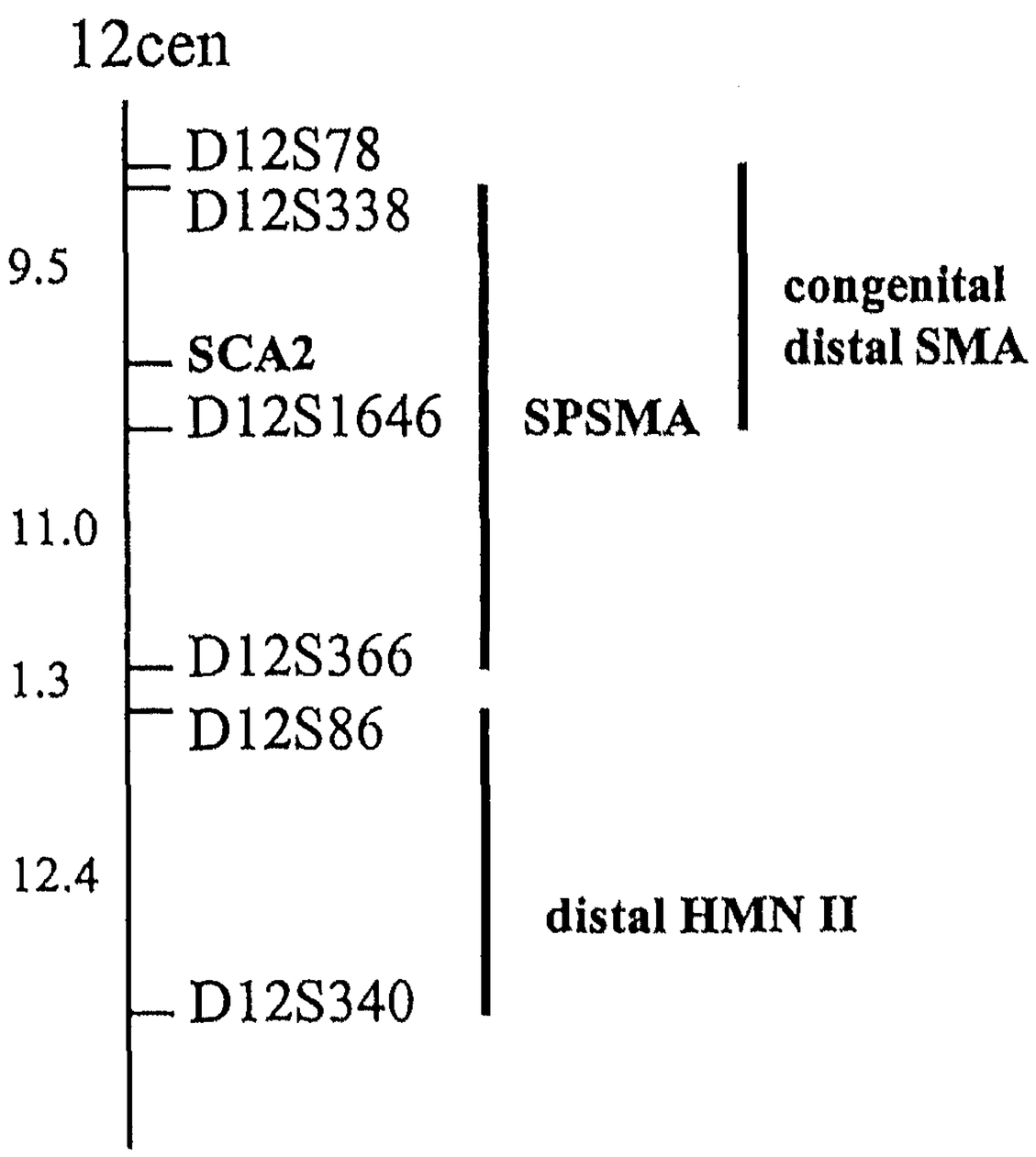

Figure 3 *Genetic map of chromosome 12q23–q24 with the location of three hereditary motor neuron diseases marked by bars. Distances are given in cM based on sex average recombination fractions. SCA2, spinocerebeller ataxia type 2.*

hereditary motor neuropathy type II (distal HMNII)[22] is located distal to the locus for the present SMA and is excluded by the markers *D12S366, D12S349* and *D12S1639* (Table 2). The locus for autosomal dominant scapuloperoneal muscular dystrophy is located proximal to the locus for the present SMA.[23]

There are no very obvious candidate genes known in the critical region of the present disorder. However, a number of genes localised in or around the interval or mapped with overlap to the critical region, can be regarded as candidates. These are, among others: protein phosphatase 1 catalytic gamma isoform (PPP1CC); protein tyrosine phosphatase non-receptor type 11 (PTPN11); nuclear transcription factor Y B-subunit (NFYB); the gene for spinocerebellar ataxia (SCA2) and mitogen activating protein kinase activated protein kinase 2 (MPAKAP kinase2),[24–26] OMIM. The nitric oxide synthase 1 (NOS1) gene, discussed as a candidate for SPSMA,[14] is located outside the critical region of the present SMA.

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